CoviDetect™

COVID-19 Mutation RT-PCR Assays

In Vitro Diagnostic Assays for Detection of Mutations found in SARS-CoV-2 B.1.1.7, B.1.351, P.1 and P.2

Instructions for use

Please read these instructions carefully before using PentaBase's CoviDetect™ COVID-19 Mutation RT-PCR Assays. It is recommended to save the *Instructions for use* for future use. Purchasers of PentaBase's CoviDetect™ COVID-19 Mutation RT-PCR Assays are only granted the right to use, but no general licensing or patent rights.

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1. Intended use

CoviDetect™ COVID-19 Mutation RT-PCR diagnostic assays are real-time RT (reverse transcription) PCR assays intended for detection of genetic variations from positive SARS-CoV-2 samples. Results are for the detection of genetic variations N501Y, P681H, Del69-70, E484K, N439K, A701V, K417N, Y453F, F476L, V553L, L18F, Del242-244, S477N, A222V, H655Y, Q677H and L452R. Genetic variants of SARS-CoV-2 RNA can be found in the liquid from upper or lower respiratory tracts of infected individuals. Samples can be obtained by nasopharyngeal or oropharyngeal swabs or from sputum or saliva. Note that infection with any variant of SARS-CoV-2 can occur without showing any symptoms.

Negative RT-PCR results do not exclude present or hinder future infection with SARS-CoV-2 virus, or any genetic variations and the result should always be combined with clinical observations, patient history, and epidemiological information.

CoviDetect™ COVID-19 Mutation RT-PCR Assays are intended for use by health professionals or qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR as well as proficient in handling biological samples.

The *Instructions for Use* or *Quick guide* is also available for download on our webpage: www.pentabase.com.

2. Summary and explanation of the assay

2.1 Indications for use

On December 31, 2019, China alerted the World Health Organization to several cases of unusual pneumonia in Wuhan. This infection has since been identified to be caused by the novel coronavirus, named SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2). COVID-19 is the first pandemic caused by coronavirus with a fast spread rate and potentially fatal infection that resulted in significant worldwide morbidity and mortality.

Viruses constantly change through mutation, which makes the emergence of new variants an expected occurrence, and accurate diagnosis of positive SARS-CoV-2 samples is important as risk related to the spread of new SARS-CoV-2 variants is of current concern. In recent months, a diversification of SARS-CoV-2 due to evolution and adaptable processes has been observed globally.

The CoviDetect™ COVID-19 Mutation RT-PCR Assay is a molecular *in vitro* diagnostic assay based melting curve analysis for detection and differentiation of SARS-CoV-2 variants in individuals. The Assays are provided in a simplex format and few multiplex configurations like for the N501Y and P681H mutations as well as for the Del69-70 and N439K mutations.

2.2. Explanation of the assay

The CoviDetect™ COVID-19 Mutation RT-PCR Assay combines real-time PCR with PentaBase's novel and selective technologies comprising both standard synthetic oligonucleotides as well as proprietary modified synthetic oligonucleotides such as EasyBeacon™ probes and SuPrimers™ for specific and sensitive amplification. The technology applies to several well dispersed real-time PCR instruments as well as PentaBase's own portfolio of instruments using standard procedures. Pentabase-modified oligos contain synthetic DNA analogues comprising a flat heteroaromatic, hydrophobic molecule and a linker. These modifications are inserted into the oligonucleotides at fixed positions during synthesis. With the use of CoviDetect™ COVID-19 Mutation RT-PCR Assays, detection of genetics variations from positive SARS-CoV-2 samples can be detected quickly, sensitively, and selectively by real-time RT-PCR followed by a DNA melt analysis.

An EasyBeacon™ probe is similar to a molecular beacon but is based on pentabase-modified oligonucleotides, which keep the probe quenched at all temperatures, without the need of an internal stem structure. This effect is due to hydrophobic interactions between the "pentabases" in the unbound probe. Another feature introduced by the pentabase-modifications is nuclease resistance. These features result in a good signal-to-noise ratio as well as a nuclease resistant probe intact for an affinity study (DNA melt analysis) after the RT PCR reaction.

SuPrimers™ are standard DNA primers modified with one or more PentaBases. Pentabases provide increased specificity and sensitivity and reduce primer-dimer formation.

2.3 Principles of the procedure

The CoviDetect™ COVID-19 Mutation RT-PCR Assays are performed on a Real-Time PCR Instrument for nucleic acid amplification and DNA melting curve analysis for detection and differentiation of SARS-CoV-2 variants in individuals. CoviDetect™ COVID-19 Mutation RT-PCR Assays are supplied as either Dispense Ready (DR) or Ready-To-Use (RTU)

versions. The Dispense Ready version includes Primer-Probe Mix and Master Mix in separate tubes to be dispensed in own plasticware before the addition of RNA. The Ready-To-Use version is pre-dispensed in 8-tube regular profile (0.2 ml) or low profile (0.1 ml) Real-Time PCR strips and needs only the addition of RNA before amplification and DNA melting curve analysis.

Note: The dispense Ready version of Del69-70/N439K multiplex includes two vials of Primer-Probe Mix and Master Mix in separate tubes.

Each of the CoviDetect™ COVID-19 Mutation RT-PCR Assays targets one viral sequence of the SARS-CoV-2 (Table 1). Amplification of SARS-CoV-2 sequence, which comprises the site of variation from the sample is achieved by using specific forward and reverse primers surrounding the site of potential variation and an EasyBeacon labelled either with FAM™, HEX™, Cy5™ or Texas Red™ spanning the site of potential variation. A heat- and inhibitor-resistant RT enzyme combined with a thermostable DNA polymerase enzyme is used for reverse transcription and subsequent amplification, and detection of amplified mutation target is achieved by melt analysis.

Table 1. List of detected regions in the CoviDetect™ COVID-19 Mutation RT-PCR Assays.

Targeted Regions	Gene	Fluorophore
N501Y	Spike Protein	HEX™
P681H	Spike Protein	Cy5™
Del69-70	Spike Protein	HEX™
E484K	Spike Protein	FAM™
N439K	Spike Protein	FAM™
A701V	Spike Protein	Cy5™
K417N	Spike Protein	Cy5™
Y453F	Spike Protein	HEX™
F476L	RNAse dependent RNA polymerase	HEX™
V553L	RNAse dependent RNA polymerase	FAM™
L18F	Spike Protein	Texas Red™
Del242-244	Spike Protein	Texas Red™
S477N	Spike Protein	Cy5™
A222V	Spike Protein	Texas Red™
H655Y	Spike Protein	FAM™
Q677H	Spike Protein	HEX™
L452R	Spike Protein	FAM™

3. Reagens and materials

The materials provided for CoviDetect™ COVID-19 Mutation RT-PCR Assays can be found in Table 2. Materials required, but not provided can be found in Table 4 and 5. Reagent handling and storage can be found in Table 3.

Refer to the section of **Reagent and materials** and **Precautions and handling requirements** for the hazard information for the products.

3.1 CoviDetect™ COVID-19 Mutation RT-PCR Assay reagents and controls

All unopened assay tubes and Master Mix must be stored as recommended in Table 3.

Table 2. List of materials provided for CoviDetect™ COVID-19 Mutation RT-PCR Assay.

CoviDetect™ COVID-19 Mutation RT-PCR Assay Dispense Ready (DR)		
Kit components	**Reagent ingredients**	**Safety symbol and warning**
COVID-19 Mutation RT-PCR Assays	Synthetic DNA	Not applicable
AmpliSmaRT™ One Step RT-qPCR Master Mix	Not applicable	EUH210 Safety data sheet available on request.
CoviDetect™ COVID-19 Mutation RT-PCR Assay Ready-To-Use (RTU)		
Kit components	**Reagent ingredients**	**Safety symbol and warning**
COVID-19 Mutation RT-PCR Assays	Synthetic DNA	Not applicable

3.2 Reagent storage and handling

The CoviDetect™ COVID-19 Mutation RT-PCR Assay is shipped with dry ice or frozen ice bricks. Reagents must be stored and handled as specified in Table 3 immediately upon arrival. The CoviDetect™ Mutation RT-PCR Assay should be stored in the original packaging and is stable at -20°C until the expiration date. Reagents should not be used past any expiration date indicated on the assay packaging. If the assay's protective packaging is damaged upon receipt or has been shipped at incorrect temperature, please contact PentaBase for instructions. Attention should be paid to the expiration date specified on the pack label. The reagents should be discarded following the disposal instructions in Section 9.

When in use, the assay components should be returned to the freezer promptly after use to minimize the time at room temperature. Repeated thawing and freezing should be kept to a minimum.

Table 3. Reagent storage and reagent expiry conditions.

Reagent	**Storage Temperature**	**Storage Time**
CoviDetect™ COVID-19 Mutation RT-PCR Assay **(DR)**	-20°C to -80°C	Stable until expiration date indicated
AmpliSmaRT™ One Step RT-qPCR Master Mix	-20°C to -80°C	Stable until expiration date indicated
CoviDetect™ COVID-19 Mutation RT-PCR Assay **(RTU)**	-20°C to -80°C	Stable until expiration date indicated

3.3 Additional materials required

Table 4. Materials and consumables required but not provided.

Material
Plasticware compatible with the PCR instrument[1]
Pipette Tips
Centrifuge for spinning tubes or plate
Nuclease free H_2O
Collection Kits
Nasopharyngeal Swab
Nasal Swab
Extraction Kit
Viral DNA/RNA Extraction Kit

3.4 Instrumentation required

Table 5. Instrumentation.

Equipment
Nucleic Acid Extraction System
Real-Time PCR instrument (three channels)

[1] Only when using Dispense Ready version

4. Precautions and handling requirements

Warnings and precautions

- For *in vitro* diagnostic use.
- Treat all biological specimens, including used CoviDetect™ COVID-19 Mutation RT-PCR Assay tubes and transfer pipettes, as if capable of transmitting infections agents. All biological specimens should be treated with universal precautions, as it is often impossible to know which specimens might be infectious.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Good laboratory practices and careful adherence to the procedures specified in these Instructions for Use are necessary. Wear laboratory coats, laboratory gloves and eye protection when handling biological samples and reagents. Laboratory gloves must be changed between handling different biological samples to avoid contamination of reagents.
- Remove gloves and wash hands thoroughly after handling samples and reagents.
- Do not use damaged CoviDetect™ COVID-19 Mutation RT-PCR Assay tube.
- Do not use a CoviDetect™ COVID-19 Mutation RT-PCR Assay pre-dispensed in a Ready-To-Use PCR tube that has been dropped while open.
- Do not open the tubes or unseal wells during or after amplification following the completion of the PCR program.
- For additional warnings, precautions, and procedures to reduce the risk of contamination for the Nucleic Acid Extraction System or Real-Time PCR Instrument consult the respective System User Guides.
- Dispose of used CoviDetect™ COVID-19 Mutation RT-PCR tube, pipette, and specimen tube according to local, state, and federal regulations for hazardous material.
- Safety Data Sheets (SDS) are available on request from your local PentaBase representative.
- Due to the high sensitivity of the assays, contamination of the work area with previous samples might cause false results. Therefore, use extreme caution not to contaminate reagents and handle samples according to standard laboratory practice.
- The reagents should not be diluted to a lower concentration than stated in the protocol. This may affect the performance of the assay.
- Do not substitute the reagents with others as it may affect the performance of the assay.
- Specimen collection must be performed using the appropriate swab types as recommended in Table 4. Inadequate or inappropriate sample collection, storage, and transport may yield incorrect or invalid results. DO NOT use cotton or calcium alginate swab, or swabs with wood shafts.
- Ensure there is no sign of leakage from the collection tube prior to running the analysis.

5. Sample collection, transport, and storage

Note: Handle all biological samples and controls as if they are capable of transmitting infectious agents.

5.1 Sample collection

The specimen should be nasopharyngeal or oropharyngeal swabs, saliva, or sputum. Preferentially, use the same sample from which SARS-CoV-2 was detected, to genotype it for mutations. Ineffective or inappropriate sample collection can result in false test results. Training in specimen collection is therefore recommended to ensure the best quality.

5.2 Transport and storage

- Transportation of collected specimens must comply with all applicable regulations for the transport of biological agents.
- Specimens can be stored in suitable buffers at 2-8°C for up to 72 hours after collection.
- If delivery and processing exceed 72 hours, the specimen should be transported or store specimens at -70°C or lower.
- Extracted RNA should always be stored at -70°C or lower in an RNase free environment.

6. Instructions for use

6.1 Procedural notes

- Do not use CoviDetect™ COVID-19 Mutation RT-PCR Assays or AmpliSmaRT™ One-Step RT-qPCR Master Mix after expiry dates.
- Do not reuse consumables. They are for one-time use only.

6.2 Reagent Preperation

6.2.1 Dispense Ready

6.2.1.1 Simplex

a. Add **10 µl** 2x AmpliSmaRT™ One-Step RT-qPCR Master Mix to each PCR tube or well.
b. Add **5 µl** 4x simplex primer/probe mix to the PCR tubes or wells.

> **Alternatively**: Dilute **2.5 µl** 8x simplex primer/probe mix 1:1 in ultra-pure or RNase free water and add to the PCR tubes or wells.

c. Add **5 µl** of the template to each PCR tube. One patient is analyzed in a single PCR tube.
d. Close all PCR tubes or seal plates.

6.2.1.2 Multiplex

a. Add **10 µl** 2x AmpliSmaRT™ One-Step RT-qPCR Master Mix to each PCR tube or well.
b. Add **5 µl** 4x Multiplex primer/probe mix to the PCR tubes or wells OR mix **2.5 µl** of each 8x simplex primer/probe mix and add to the PCR tubes or wells.
c. Add **5 µl** of the template to each PCR tube. One patient is analyzed in a single PCR tube.
d. Close all PCR strips or seal plates.

6.2.2 Ready-To-Use

a. Spin down the PCR tubes or plates before the adding of the template to ensure that all reagents are collected at the bottom.
b. Add **5 µl** of the template to each PCR tube or well in the plate. One patient is analyzed in a single PCR tube or well.
c. Close all PCR tubes or seal plates.

6.2.3 SARS CoV-2 RNA control

When running the melting analysis, we recommend a SARS CoV-2 wild-type RNA control to be included. 200 µl (20 copies/µl) should be added during the RNA extraction procedure.

6.3 Running CoviDetect™ COVID-19 Mutation RT-PCR Assays

a. Spin down the PCR tubes or plates (1-2 minutes at 4000-5000 rpm) to ensure that all reagents are collected at the bottom of the strips or wells and to eliminate air bubbles in the mixes.
b. Place the PCR tubes or plate in the Real-Time PCR instrument and run the program listed in Table 6.

Table 6. RT-PCR protocol for running CoviDetect™ COVID-19 Mutation RT-PCR Assays.

Protocol	Temperature [°C]	Time	Cycles	Channel
Reverse transcription	52	5 min	1	-
Hold	95	30 sec	1	-
Cycling	94	15 sec	45	HEX™ (538nm/551nm) Cy5™ (647nm/665nm) FAM™ (495nm/516nm) Texas Red™ (596nm/615nm) Measured fluorescence intensity during annealing/elongation (60°C)
	60	45 sec		
Hold	95	60 sec	1	-
Melting	40	60 sec	1	HEX™ (538nm/551nm) Cy5™ (647nm/665nm) FAM™ (495nm/516nm) Texas Red™ (596nm/615nm) Measured fluorescence intensity during melting
	Up to 80	10 readings/°C ***Note**		

***Note**: For CFX use 2 reading/°C.

7. Data Analysis

The melting temperature and graphs for the CoviDetect™ COVID-19 Mutation RT-PCR Assays, and how to analyze the data are listed in the following sections. It is not possible to determine the genotype in case there is no melting curve of the sample. If more specimen is available, repeat the extraction and run the test again. If all markers remain negative after repeating the test, no diagnosis can be concluded, and if possible, a new specimen should be collected for testing or the sample should be sent for sequencing.

7.1 Interpretation of results

7.1.1 Score Chart

An overview of the possible outcomes of the different CoviDetect COVID-19 Mutation Assays is shown in Table 7.

Table 7. Analysis outcomes based on target melting curves. Conclusions are based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

	B.1.1.7	B.1.351	Mink	N439K	P.1	P.2	Remdesivir Ineffectivity	B.1.525	B.1.427/B.1.429
L18F	-	-	-	-	+		-	-	-
Del69-70	+	-	+	+/-	-		-	+	-
DelY144	+	-	-	-	-		-	+	-
A222V	-	-	-	-	-		-	-	-
Del242-244	-	+	-	-	-		-	-	-
K417N	-	+	-	-	T		-	-	-
N439K	-	-	-	+	-		-	-	-
L452R	-	-	-	-	-	-	-	-	+
Y453F	-	-	+	-	-		-	-	-
S477N	-	-	+	-	-		-	-	-
E484K	-	+	-	-	+	+	-	+	-
N501Y	+	+	-	-	+		-	-	-
H655Y	-	-	-	-	+		-	-	-
Q677H	-	-	-	-	-	-	-	+	-
P681H	+	-	-	-	-		-	-	-
A701V	-	+	-	-	-		-	-	-
F476L	-	-	-	-	-		+	-	-
V553L	-	-	-	-	-		+	-	-

7.1.2 BaseTyper™

7.1.2.1 CoviDetect™ COVID-19 N501Y Simplex Assay

Table 8. Analysis outcomes based on target melting curves on BaseTyper™. Genotype is based on target melting curve compared to a WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>62.0	HEX™	The sample is positive for the N501Y mutation
56.0-60.5	HEX™	The sample is negative for the N501Y mutation
<56.0	HEX™	The sample is negative for the N501Y mutation, but most likely contain a different mutation in the probe area.
No peaks	HEX™	Not possible to determine genotype

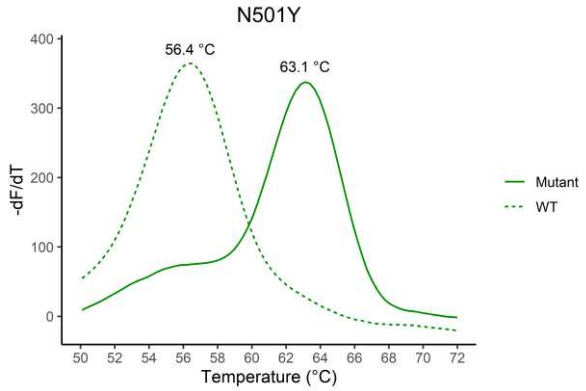

Figure 1. The melting curve of a sample positive for N501Y analysed on the BaseTyper™. The N501Y mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.2.2 CoviDetect COVID-19 P681H Simplex Assay

Table 9. Analysis outcomes based on target melting curves on BaseTyper™. Conclusions are based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>64.0	Cy5™	The sample is positive for the P681H mutation
55.0-58.5	Cy5™	The sample is negative for the P681H mutation
<55.0	Cy5™	The sample is negative for the P681H mutation, but most likely contain a different mutation in the probe area.
No peaks	Cy5™	Not possible to determine genotype

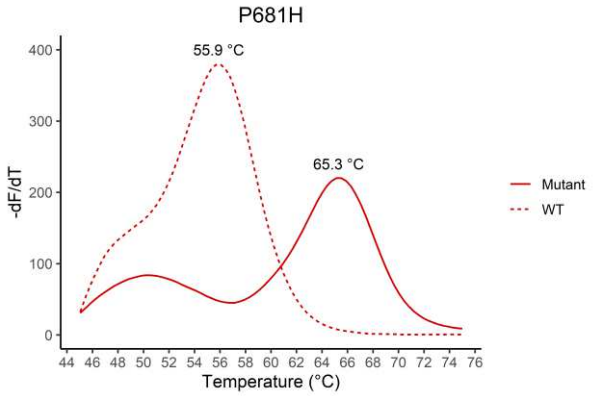

Figure 2. The melting curve of a sample positive for P681H analysed on the BaseTyper™. The P681H mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.2.3 CoviDetect COVID-19 Del69-70 Simplex Assay

Table 10. Analysis outcomes based on target melting curves on BaseTyper™. Conclusions are based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>65.5	HEX™	The sample is positive for the Del69-70 mutation
57.5-60.0	HEX™	The sample is negative for the Del69-70 mutation
<57.5	HEX™	The sample is negative for the Del69-70 mutation, but most likely contain a different mutation in the probe area.
No peaks	HEX™	Not possible to determine genotype

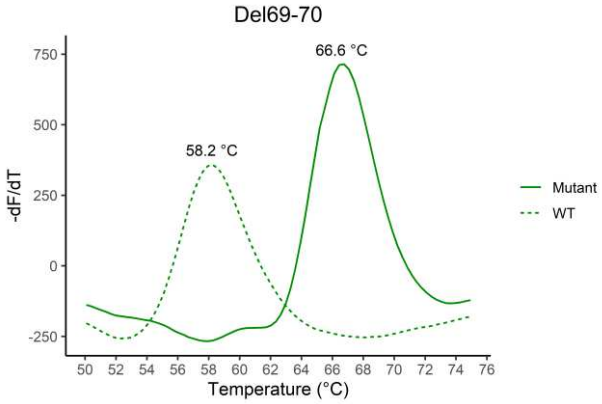

Figure 3. The melting curve of a sample positive for del69-70 analysed on the BaseTyper™. The deletion 69-70 has a higher affinity for the probe and will melt at a higher temperature.

7.1.2.4 CoviDetect™ COVID-19 E484K Simplex Assay

Table 11. Analysis outcomes based on target melting curves on the BaseTyper™. Conclusions are based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>60.0	FAM™	The sample is positive for the E484K mutation
52.0-55.0	FAM™	The sample is negative for the E484K mutation
<52.0	FAM™	The sample is negative for the E484K mutation, but most likely contain a different mutation in the probe area.
No peaks	FAM™	Not possible to determine genotype

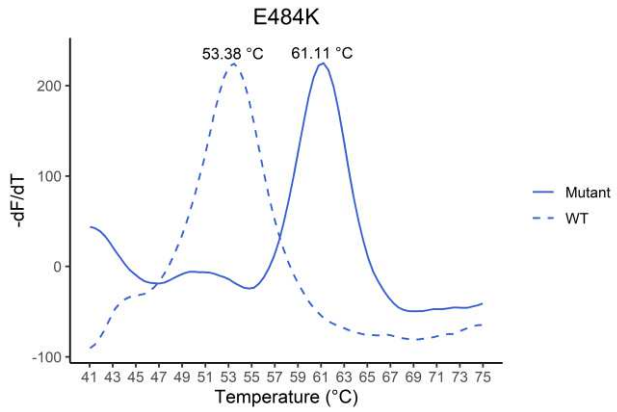

Figure 4. The melting curve of a sample positive for E484K analysed on the BaseTyper™. The E484K mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.2.5 CoviDetect™ COVID-19 N439K Simplex Assay

Table 12. Analysis outcomes based on target melting curves on BaseTyper™. Conclusions are based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>58.0	FAM™	The sample is positive for the N439K mutation
52.5-55.5	FAM™	The sample is negative for the N439K mutation
<52.5	FAM™	The sample is negative for the N439K mutation, but most likely contain a different mutation in the probe area.
No peaks	FAM™	Not possible to determine genotype

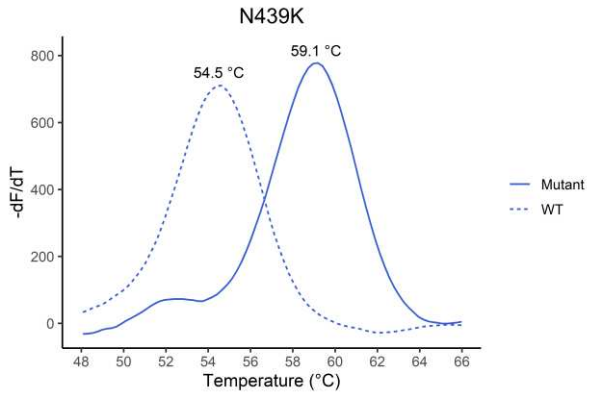

Figure 5. The melting curve of a sample positive for N439K analysed on the BaseTyper™. The N439K mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.2.6 CoviDetect™ COVID-19 A701V Simplex Assay

Table 13. Analysis outcomes based on target melting curves on the BaseTyper™. Genotype is based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>67.0	Cy5™	The sample is positive for the A701V mutation
62.0-65.5	Cy5™	The sample is negative for the A701V mutation
<62.0	Cy5™	The sample is negative for the A701V mutation, but most likely contain a different mutation in the probe area.
No peaks	Cy5™	Not possible to determine genotype

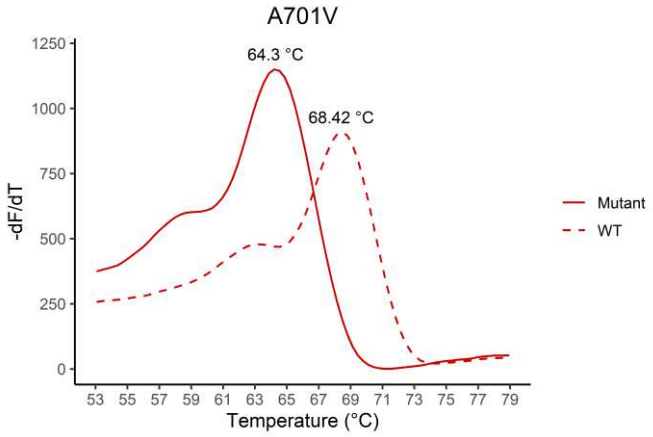

Figure 6. The melting curve of a sample positive for A701V analysed on the BaseTyper™. The A701V mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.2.7 CoviDetect™ COVID-19 Y453F Simplex Assay

Table 14. Analysis outcomes based on target melting curves on the BaseTyper™. Genotype is based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>62.5	HEX™	The sample is positive for the Y453F mutation
57.5-61.5	HEX™	The sample is negative for the Y453F mutation
<57.5	HEX™	The sample is negative for the Y453F mutation, but most likely contain a different mutation in the probe area.
No peaks	HEX™	Not possible to determine genotype

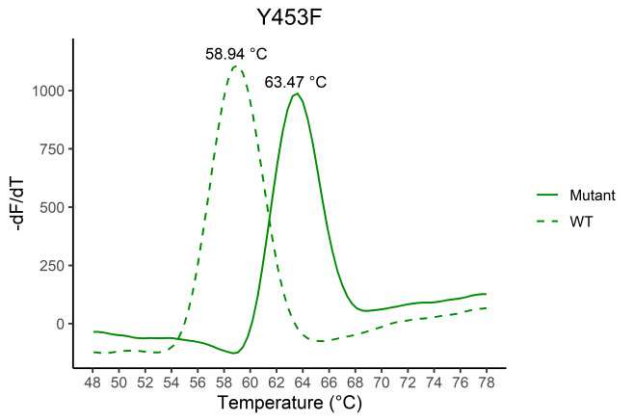

Figure 7. The melting curve of a sample positive for Y453F analysed on the BaseTyper™. The Y453F mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.2.8 CoviDetect™ COVID-19 F476L Simplex Assay

Table 15. Analysis outcomes based on target melting curves on the BaseTyper™. Genotype is based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>63.0	HEX™	The sample is positive for the F476L mutation
57.5-61.5	HEX™	The sample is negative for the F476L mutation
<57.5	HEX™	The sample is negative for the F476L mutation, but most likely contain a different mutation in the probe area.
No peaks	HEX™	Not possible to determine genotype

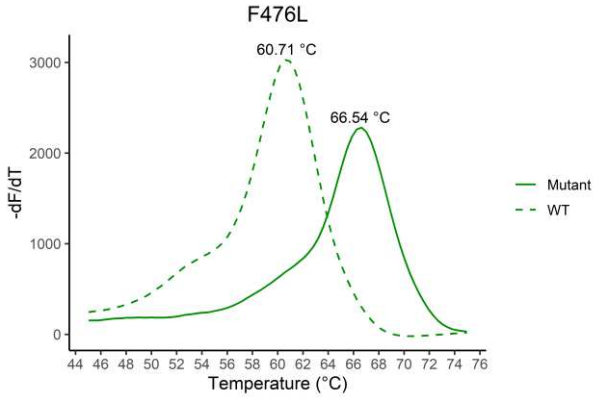

Figure 8. The melting curve of a sample positive for F476L analysed on the BaseTyper™. The Y453F mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.2.9 CoviDetect™ COVID-19 V553L Simplex Assay

Table 16. Analysis outcomes based on target melting curves on the BaseTyper™. Genotype is based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>65.5	FAM™	The sample is positive for the V553L mutation
61.5-64.0	FAM™	The sample is negative for the V553L mutation
<61.5	FAM™	The sample is negative for the V553L mutation, but most likely contain a different mutation in the probe area.
No peaks	FAM™	Not possible to determine genotype

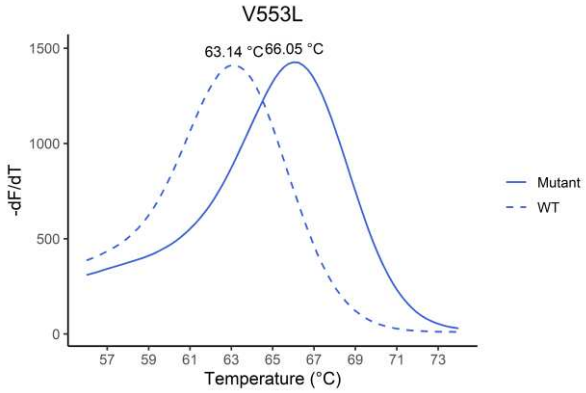

Figure 9. The melting curve of a sample positive for V553L analysed on the BaseTyper™. The V553L mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.2.10 CoviDetect™ COVID-19 L18F Simplex Assay

Table 17. Analysis outcomes based on target melting curves on the BaseTyper™. Genotype is based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>63.0	Texas Red™	The sample is positive for the L18F mutation
56.5-58.5	Texas Red™	The sample is negative for the L18F mutation
<56.5	Texas Red™	The sample is negative for the L18F mutation, but most likely contain a different mutation in the probe area.
No peaks	Texas Red™	Not possible to determine genotype

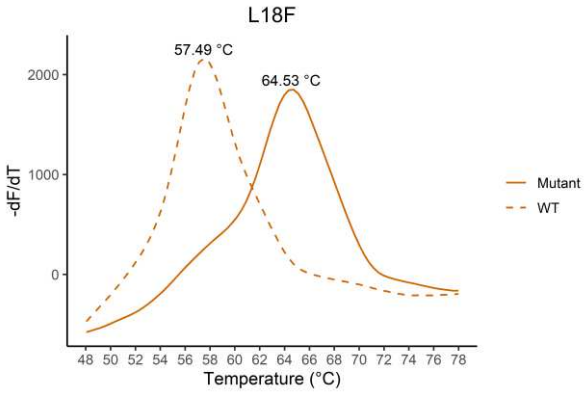

Figure 10. The melting curve of a sample positive for L18F analysed on the BaseTyper™. The L18F mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.2.11 CoviDetect™ COVID-19 Del242-244 Simplex Assay

Table 18. Analysis outcomes based on target melting curves on the BaseTyper™. Genotype is based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>67.0	Texas Red™	The sample is positive for the Del242-244 mutation
61.0-64.0	Texas Red™	The sample is negative for the Del242-244 mutation
<61.0	Texas Red™	The sample is negative for the Del242-244 mutation, but most likely contain a different mutation in the probe area.
No peaks	Texas Red™	Not possible to determine genotype

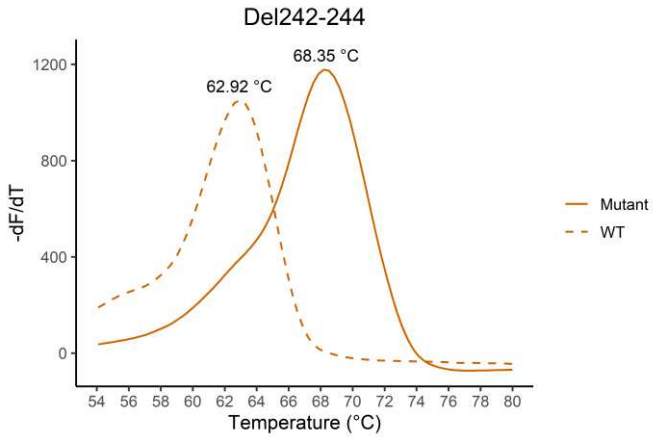

Figure 11. The melting curve of a sample positive for Del242-244 analysed on the BaseTyper™. The Del242-244 mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.2.12 CoviDetect™ COVID-19 K417N Simplex Assay

Table 19. Analysis outcomes based on target melting curves on the BaseTyper™. Genotype is based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>63.5	Cy5™	The sample is positive for the K417N mutation
59.0-61.5	Cy5™	The sample is negative for the K417N mutation
55.0-56.5	Cy5™	The sample is most likely positive for K417T mutation
<55.0	Cy5™	The sample is negative for the K417N mutation, but most likely contain a different mutation in the probe area.
No peaks	Cy5™	Not possible to determine genotype

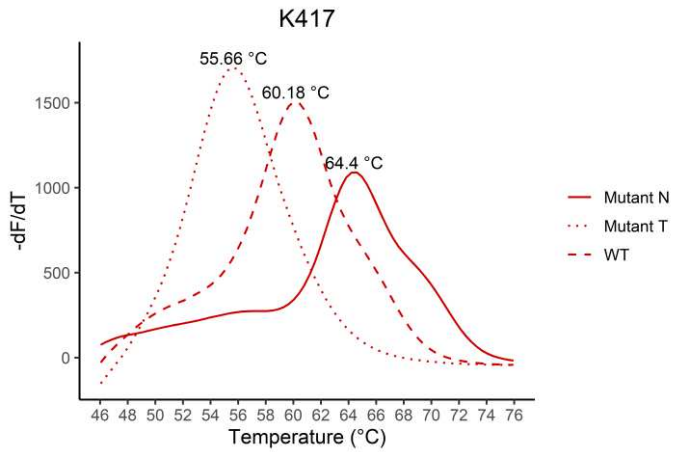

Figure 12. The melting curve of a sample positive for K417N or K417T analysed on the BaseTyper™. The mutations have a higher affinity for the probe and will melt at a higher temperature.

7.1.2.13 CoviDetect™ COVID-19 A222V Simplex Assay

Table 20. Analysis outcomes based on target melting curves on the BaseTyper™. Genotype is based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>64.5	Texas Red™	The sample is positive for the A222V mutation
59.0-61.5	Texas Red™	The sample is negative for the A222V mutation
<59.0	Texas Red™	The sample is negative for the A222V mutation, but most likely contain a different mutation in the probe area.
No peaks	Texas Red™	Not possible to determine genotype

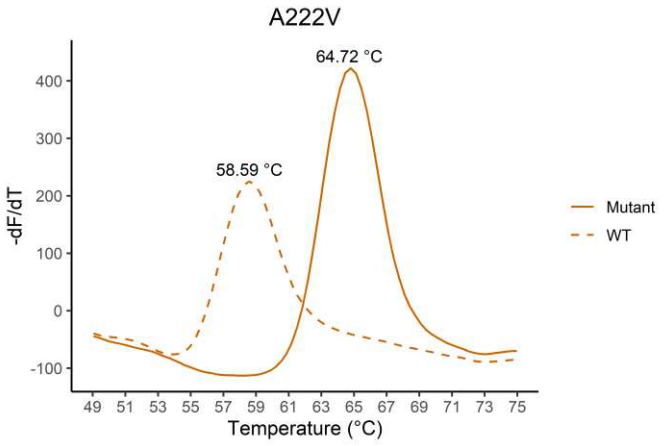

Figure 13. The melting curve of a sample positive for A222V analysed on the BaseTyper™. The A222V mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.2.14 CoviDetect™ COVID-19 H655Y Simplex Assay

Table 21. Analysis outcomes based on target melting curves on the BaseTyper™. Genotype is based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>66.5	FAM™	The sample is positive for the H655Y mutation
62.0-65.5	FAM™	The sample is negative for the H655Y mutation
<62.0	FAM™	The sample is negative for the H655Y mutation, but most likely contain a different mutation in the probe area.
No peaks	FAM™	Not possible to determine genotype

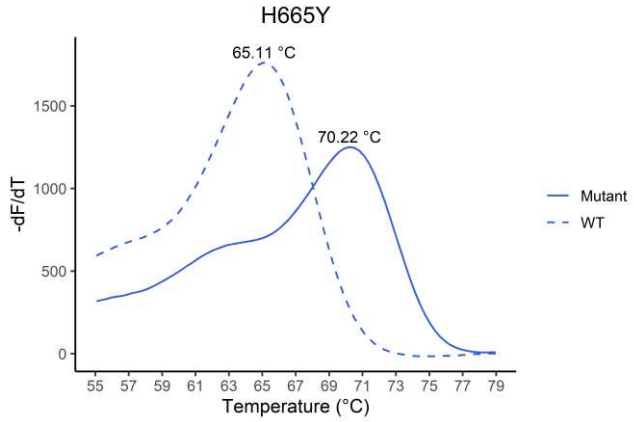

Figure 14. The melting curve of a sample positive for H655Y analysed on the BaseTyper™. The H655Y mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.2.15 CoviDetect™ COVID-19 S477N Simplex Assay

Table 22. Analysis outcomes based on target melting curves on the BaseTyper™. Genotype is based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>65.0	Cy5™	The sample is positive for the S477N mutation
59.5-61.5	Cy5™	The sample is negative for the S477N mutation
<59.5	Cy5™	The sample is negative for the S477N mutation, but most likely contain a different mutation in the probe area.
No peaks	Cy5™	Not possible to determine genotype

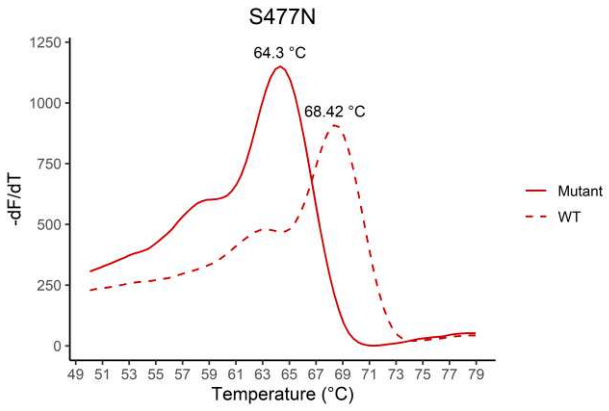

Figure 15. The melting curve of a sample positive for S477N analysed on the BaseTyper™. The S477N mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.2.7 CoviDetect™ COVID-19 Q677H Simplex Assay

Table 23. Analysis outcomes based on target melting curves on the BaseTyper™. Genotype is based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>62.0	HEX™	The sample is positive for the Q677H mutation
59.0-61.5	HEX™	The sample is negative for the Q677H mutation
<59.0	HEX™	The sample is negative for the Q677H mutation, but most likely contain a different mutation in the probe area.
No peaks	HEX™	Not possible to determine genotype

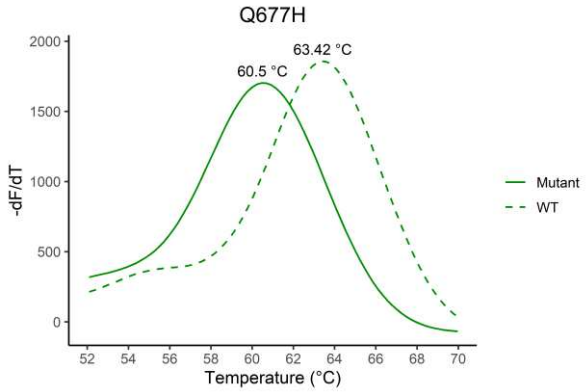

Figure 16. The melting curve of a sample positive for Q677H analysed on the BaseTyper™ . The N501Y mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.2.8 CoviDetect™ COVID-19 L452R Simplex Assay

Table 24. Analysis outcomes based on target melting curves on the BaseTyper™. Genotype is based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>60.5	FAM™	The sample is positive for the L452R mutation
56.5-59.5	FAM™	The sample is negative for the L452R mutation
<59.5	FAM™	The sample is negative for the L452R mutation, but most likely contain a different mutation in the probe area.
No peaks	FAM™	Not possible to determine genotype

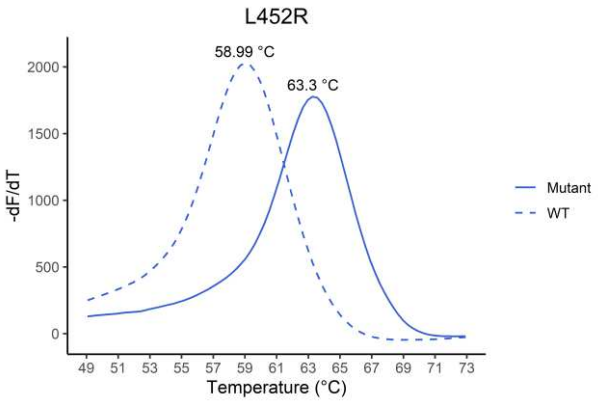

Figure 17. The melting curve of a sample positive for L452R analysed on the BaseTyper™. The L452R mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.3 LightCycler® 480 Instrument II

7.1.3.1 CoviDetect™ COVID-19 N501Y Simplex Assay

Table 25. Analysis outcomes based on target melting curves on LightCycler® 480 Instrument II. Genotype is based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>60.5	HEX™	The sample is positive for the N501Y mutation
55.0-57.0	HEX™	The sample is negative for the N501Y mutation
<55.0	HEX™	The sample is negative for the N501Y mutation, but most likely contain a different mutation in the probe area.
No peaks	HEX™	Not possible to determine genotype

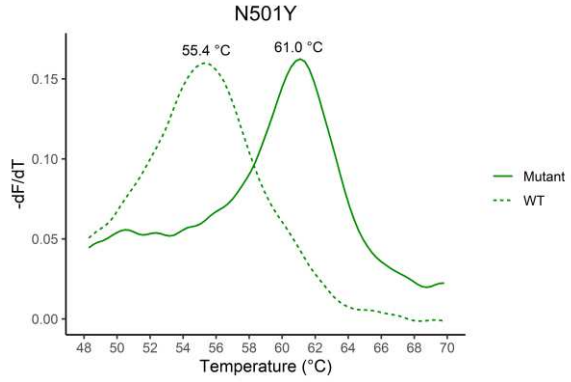

Figure 18. The melting curve of a sample positive for N501Y analysed on the LightCycler®480 Instrument II. The N501Y mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.3.2 CoviDetect™ COVID-19 P681H Simplex Assay

Table 26. Analysis outcomes based on target melting curves on LightCycler® 480 Instrument II. Conclusions are based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>62.0	Cy5™	The sample is positive for the P681H mutation
55.0-57.0	Cy5™	The sample is negative for the P681H mutation
<55.0	Cy5™	The sample is negative for the P681H mutation, but most likely contain a different mutation in the probe area.
No peaks	Cy5™	Not possible to determine genotype

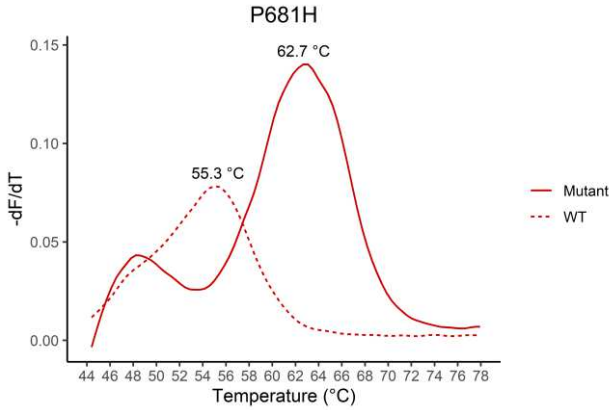

Figure 19. The melting curve of a sample positive for P681H analysed on the LightCycler® 480 Instrument II. The P681H mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.3.3 CoviDetect™ COVID-19 P681H Simplex Assay

Table 27. Analysis outcomes based on target melting curves on LightCycler® 480 Instrument II. Conclusions are based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>63.5	HEX™	The sample is positive for the Del69-70 mutation
54.5-56.5	HEX™	The sample is negative for the Del69-70 mutation
<54.5	HEX™	The sample is negative for the Del69-70 mutation, but most likely contain a different mutation in the probe area.
No peaks	HEX™	Not possible to determine genotype

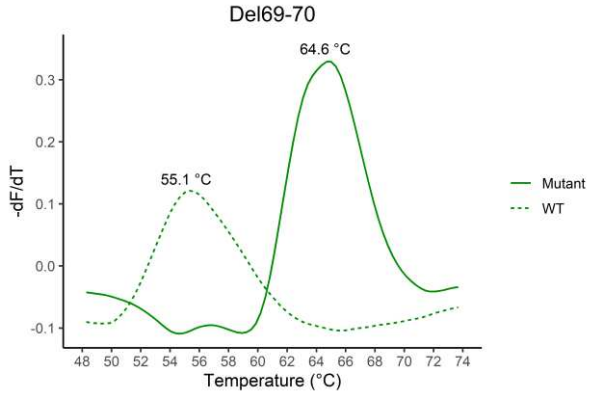

Figure 20. The melting curve of a sample positive for del69-70 analysed on the LightCycler® 480 Instrument II. The deletion 69-70 has a higher affinity for the probe and will melt at a higher temperature.

7.1.3.4 CoviDetect™ COVID-19 E484K Simplex Assay

Table 28. Analysis outcomes based on target melting curves on the LigthCycler® 480 Instrument II. Conclusions are based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>58	FAM™	The sample is positive for the E484K mutation
51.0-53.0	FAM™	The sample is negative for the E484K mutation
<51.0	FAM™	The sample is negative for the E484K mutation, but most likely contain a different mutation in the probe area.
No peaks	FAM™	Not possible to determine genotype

7.1.3.5 CoviDetect™ COVID-19 N439K Simplex Assay

Table 29. Analysis outcomes based on target melting curves on LightCycler® 480 Instrument II. Conclusions are based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>56.5	FAM™	The sample is positive for the N439K mutation
52.0-54.0	FAM™	The sample is negative for the N439K mutation
<52.0	FAM™	The sample is negative for the N439K mutation, but most likely contain a different mutation in the probe area.
No peaks	FAM™	Not possible to determine genotype

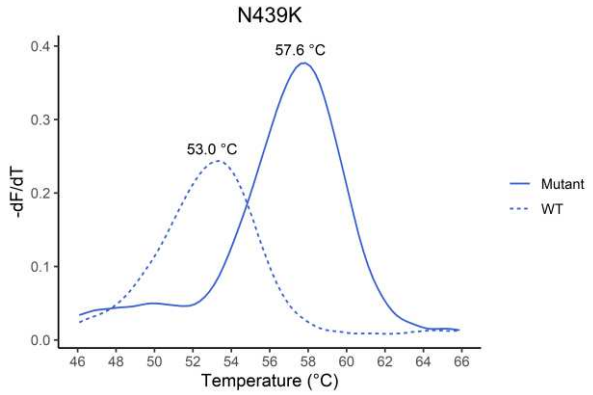

Figure 21. The melting curve of a sample positive for N439K analysed on the LigthCycler® 480 Instrument II. The N439K mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.4 CFX

7.1.4.1 CoviDetect™ COVID-19 N501Y Simplex Assay

Table 30. Analysis outcomes based on target melting curves on CFX. Genotype is based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>60.5	HEX™	The sample is positive for the N501Y mutation
54.5-58.5	HEX™	The sample is negative for the N501Y mutation
<54.5	HEX™	The sample is negative for the N501Y mutation, but most likely contain a different mutation in the probe area.
No peaks	HEX™	Not possible to determine genotype

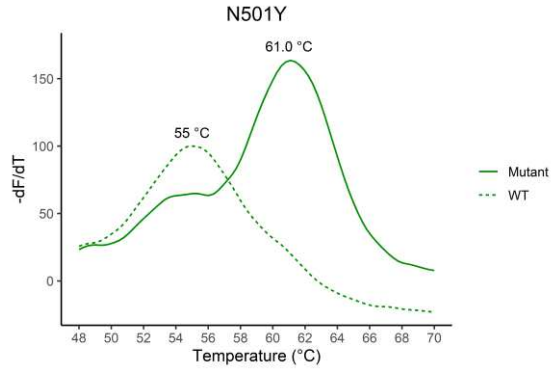

Figure 22. The melting curve of a sample positive for N501Y analysed on the CFX96. The N501Y mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.4.2 CoviDetect™ COVID-19 P681H Simplex Assay

Table 31. Analysis outcomes based on target melting curves on CFX. Conclusions are based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>62.5	Cy5™	The sample is positive for the P681H mutation
53.0-57.0	Cy5™	The sample is negative for the P681H mutation
<53.0	Cy5™	The sample is negative for the P681H mutation, but most likely contain a different mutation in the probe area.
No peaks	Cy5™	Not possible to determine genotype

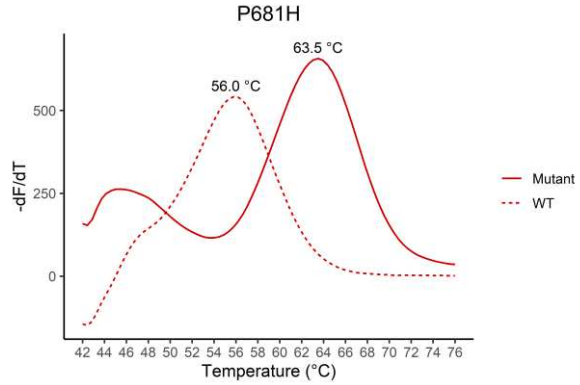

Figure 23. The melting curve of a sample positive for P681H analysed on the CFX. The P681H mutation has a higher affinity for the probe and will melt at a higher temperature.

7.1.4.3 CoviDetect™ COVID-19 Del69-70 Simplex Assay

Table 32. Analysis outcomes based on target melting curves on CFX. Conclusions are based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>64.0	HEX™	The sample is positive for the Del69-70 mutation
55.0-59.0	HEX™	The sample is negative for the Del69-70 mutation

<55.0	HEX™	The sample is negative for the Del69-70 mutation, but most likely contain a different mutation in the probe area.
No peaks	HEX™	Not possible to determine genotype

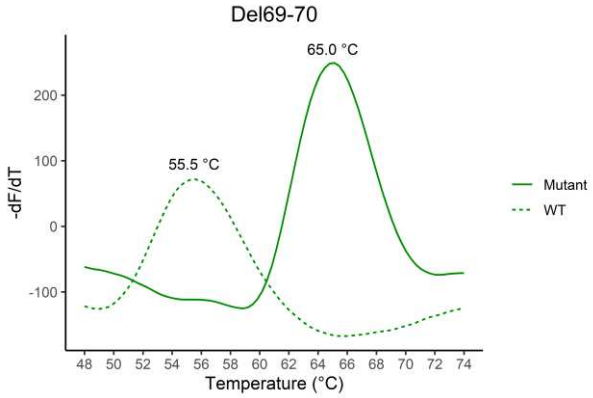

Figure 24. The melting curve of a sample positive for del69-70 analysed on the CFX. The deletion 69-70 has a higher affinity for the probe and will melt at a higher temperature.

7.1.4.4 CoviDetect™ COVID-19 E484K Simplex Assay

Table 33. Analysis outcomes based on target melting curves on the CFX. Conclusions are based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>58	FAM™	The sample is positive for the E484K mutation
51.0-54.0	FAM™	The sample is negative for the E484K mutation
<51.0	FAM™	The sample is negative for the E484K mutation, but most likely contain a different mutation in the probe area.
No peaks	FAM™	Not possible to determine genotype

7.1.4.5 CoviDetect™ COVID-19 N439K Simplex Assay

Table 34. Analysis outcomes based on target melting curves on LightCycler® 480 Instrument II. Conclusions are based on target melting curve compared to an WT SARS-Co-V-2 RNA control.

Melting peak [°C]	Channel	Conclusion
>57.5	FAM™	The sample is positive for the N439K mutation
52.5-56.5	FAM™	The sample is negative for the N439K mutation
<52.5	FAM™	The sample is negative for the N439K mutation, but most likely contain a different mutation in the probe area.
No peaks	FAM™	Not possible to determine genotype

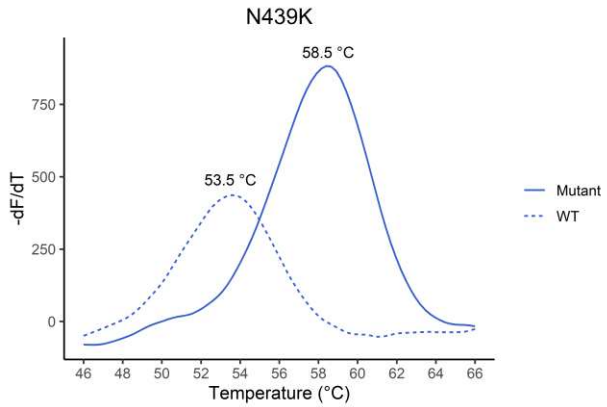

Figure 25. The melting curve of a sample positive for N439K analysed on the CFX. The N439K mutation has a higher affinity for the probe and will melt at a higher temperature.

8. Limitations

- A negative test result does not exclude infection with SARS-CoV-2, and treatment of a patient should not exclusively be based on the test result. Multiple specimens collected at different times from the same patient may be necessary to detect the virus since it is unknown when the viral levels in the body will peak.
- Incorrect collection, transportation, or handling of the sample could cause false-negative test results. Also, a very low amount of virus RNA in the specimen or amplification inhibitors could give false-negative test results.
- Do not use reagents that have expired.
- The assays cannot exclude that the patient is infected with other viruses or bacteria.

9. Disposal

The disposal of unused kit reagents, biological samples and post-amplified PCR tubes or plates according to local, state and federal regulations.

10. Manufacturer and distributors

For technical assistance in Denmark please contact PentaBase ApS:
Petersmindevej 1A
DK-5000 Odense, Denmark

Telephone: (+45) 36 96 94 96
Email: support@pentabase.com
Webpage: www.pentabase.com
For technical assistance in all other countries, contact your local distributor. A complete list of distributors is available at www.pentabase.com.